

# **Structural Studies of Soft Matter Systems by Resonant Scattering of X-rays - Application to Chiral and Non-chiral Liquid Crystals**

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Resonant scattering of x-rays has proved to be a unique and very powerful technique to characterize helical symmetries in the microscopic structure of complex fluids with low dimensionality order, such as smectic liquid crystals. The tensor symmetry of the structure factor allows to extend structural analysis beyond the limits of conventional crystallography. The aim of this presentation is to illustrate the capability of the technique in Soft Matter materials. Recent results obtained in chiral and non-chiral liquid crystals will be reviewed. In the case of antiferroelectric Smectic C phases and related chiral sub-phases, several kinds of helical arrangements have been unambiguously characterized. More recently, a new superstructure has been detected in mysterious smectic phases made of non chiral V-shaped molecules (so-called banana molecules). The different experimental conditions tested so far will also be reviewed (choice of resonant atom, scattering geometry, sample geometry, polarization detection) in order to define guidelines for future studies.

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## **Design and Structural Characterization of Artificial Metalloprotein Maquettes Vectorially-Oriented at an Interface**

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Bundles of  $\alpha$ -helices provide a scaffold for binding prosthetic groups at selected locations within the structure to mimic functions exhibited by biological proteins. The first designed artificial peptides, or "maquettes", used amphipathic di-helices which self-assembled in aqueous solution forming 4-helix bundles. To realize any device applications, the peptides must be vectorially-oriented in an ensemble, e.g., at an interface. Previously, the di-helices were made amphiphilic via attachment of C16 hydrocarbon chains to their N-termini and shown to be vectorially-oriented at an air-water interface with their helical axes normal to the interface at higher surface pressures via specular X-ray reflectivity. Any vectorial function exhibited by the peptide's prosthetic groups, e.g., light-induced electron transfer, would necessarily occur only on one side of the interface. Here, each  $\alpha$ -helix is again designed to be amphipathic with polar and nonpolar faces, but the sequence was such that if the nonpolar faces of each amphipathic helix were apposed in the di-helix over the first  $n$  residues of each helix, the polar faces would be apposed over the last  $m$  residues. Nonresonance specular X-ray reflectivity has shown that these di-helices vectorially orient at the air-water interface for the pure peptide at higher surface pressures and in binary mixtures with a phospholipid at much lower surface pressures. This allows positioning of prosthetic groups on either side of the interface. Resonance specular X-ray reflectivity, off-specular X-ray reflectivity and neutron reflectivity can be used to determine key aspects of the secondary, tertiary and quaternary structures of these maquettes vectorially-oriented at an interface.

## **Hard Core Models for Tobacco Mosaic Virus Liquid Crystals Ordered by Soft Electrostatic Repulsion**

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Tobacco mosaic virus (TMV) is the paradigmatic liquid-crystal forming hard-rod system. Rod lengths range from the 3,000Å of the monomeric particle to indefinitely long in end-to-end aggregated specimens. Effective packing diameters range from 150Å for the interlocked 180Å wide helical particles in dry columnar crystalline specimens to several thousand Å in dilute specimens of end-to-end aggregated particles at very low ionic strength. The characteristic liquid-crystalline properties of TMV were established by the pioneering optical and x-ray diffraction studies of Bernal and Fankuchen over 60 years ago. They surmised that the nematic phase was a less-ordered swollen version of the columnar crystalline phase, but small-angle equatorial diffraction measurements of the interparticle interference demonstrate the liquid-like lateral order in the nematic phase.

The interference function has been measured, in the range of Bragg spacings from ~1,000Å to ~50Å, by dividing the diffracted intensity by the square of the single particle structure factor amplitude obtained by back transforming the radial density profile of the particle refined against higher resolution data. The first peak and succeeding minimum and maximum of the interference function measured for nematic specimens at concentrations of 1 - 25% can be accurately fit by the theoretical "structure factor" calculated from the two-dimensional, hard-core pair-distribution function by the method of Baus and Colot. The two parameters required for this fitting are the effective particle diameter, determined by electrostatic repulsion, and the packing fraction, determined by the concentration. As a function of the product of the scattering vector and the packing diameter, the position of the zeros of the interference function on the high-angle side of the maxima for the hard-core model are nearly independent of the packing fraction, thus, fitting the experimental data to these zeros defines the effective diameter. The packing fraction is then determined by fitting the calculated shape of the interference function to the data. The packing diameter depends sensitively on the ionic strength of the solution, which determines the Debye screening length, but is insensitive to pH in the range 4.5 - 8, since counter ion collapse maintains a near constant surface potential. For specimens at concentrations of 15 - 25% with variable amounts of salt, packing diameters in the range 220 - 310Å have been measured, and for dilute end-to-end aggregated particles at concentrations of 1 - 2.5% and ionic strength as low as 1mM, diameters up to 700Å have been measured. The smaller packing diameters measured for nematic specimens are close to the values of hexagonal lattice constants measured for colloidal crystalline specimens.

## **Time-Resolved Structural Biology of Macromolecules Using Synchrotron Footprinting**

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Using a novel technology called Synchrotron X-ray Footprinting we can probe the specific interactions of DNA and DNA binding proteins at single basepair resolution on millisecond and longer timescales. Single stranded RNA molecules can fold into unique three-dimensional structures that play key roles in RNA functions such as protein binding and catalysis. We have used the footprinting method to study the folding of the Tetrahymena ribozyme with unprecedented structural resolution on millisecond timescales. In addition, interactions of transcription factors and DNA are studied with fine details on fast time resolutions.

Synchrotron footprinting has also been developed to analyze protein folding and protein-protein interactions. The system of interest is the actin cytoskeleton. Elucidating the mechanisms underlying the dynamic behavior of the actin cytoskeleton is essential to understanding cell locomotion, cytokinesis, and complex morphogenetic and developmental programs in multicellular organisms. These same mechanisms underlie pathological states such as neoplastic transformation and tumor metastasis and the invasive and motile mechanism used by *Listeria* and *Shigella* and a range of enteropathic bacteria. Using novel footprinting methods we are examining the severing processes undertaken by cofilin and gelsolin in their interactions with actin monomers and filaments. These studies will give an intimate portrait of the dynamics of cytoskeletal assembly in the cell.

## ***In-situ Structural Evolution During Fiber Processing***

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The structure and morphology of spun polymer fibers are highly dependent on the spinning conditions used during processing. The filament that undergoes changes developed between the spinneret and the take-up device can often determine its mechanical performance.

Exceptionally high tensile properties can be attained in fully extended rigid polymer chains, such as poly-*p*-phenylenebenzobisoxazole (PBO). It has been demonstrated that in PBO fibers, high modulus can be retained even at elevated temperatures. The Young's modulus of the PBO fiber has been reported to be as high as 350 GPa, which is significantly greater than that of poly-*p*-phenylene terephthalamide (PPTA or Kevlar) fiber.

The general method for fabrication of several high-strength fibers, such as PBO and Kevlar, starts with a dry-jet wet-spinning process from a polymer solution. The solvents used for processing PBO and Kevlar fibers are, respectively, polyphosphoric acid (PPA) and concentrated sulfuric acid. With a PBO molecular weight of  $\geq 10,000$  g/mol and a concentration of around 13-15% in PPA (often called "dope"), the solution extruded from the spinneret can develop lyotropic liquid crystalline characteristics. Fibers drawn from such a solution can achieve a high degree of orientation. A complex coagulation process has a dominating effect on the final fiber structure. In the coagulation process, the solvent, PPA, as in the case of PBO, is leached out by water, often at elevated temperatures. The interactions are affected by the structural development perpendicular to the fiber axis and therefore the structures of the core and of the skin of the fiber are usually not the same. Post processing, such as heat-treatment under tension, can further enhance the fiber modulus. Thus, it is very interesting and important to study the changes of structure and morphology *in situ* during the spinning and the coagulation process.

In situ SAXS/WAXD results during PBO solution spinning at the X27C Advanced Polymers Beamline of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (BNL) are presented in terms of different stages of processing from (1) before coagulation (2) after coagulation (3) as-spun fiber, to (4) heat-treated fiber. Quantitative results of the structural and morphological changes in the fiber under different spinning conditions are discussed.

### **Acknowledgements**

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## **Structure and Fluctuation of Liquid Interfaces and Membranes**

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I will present in this talk some examples of grazing incidence diffuse x-ray scattering studies of liquid surfaces and interfaces. This technique allows a precise determination of the interface structure and fluctuations down to molecular length-scales. I will first show how van der Waals forces affect the liquid-vapour interface at the nanometer scale, increasing its roughness. At length-scales of the order of the bulk correlation length, fluctuations are limited by an intrinsic bending rigidity. This scale-dependant surface tension is well described using a recent density functionnal theory for the different investigated liquids. Monomolecular films decrease the surface tension, and the enhancement of fluctuations can be dramatic at liquid-liquid interfaces or for bilayer lipid membranes in water. On the other hand, the film bending rigidity can almost suppress fluctuations. I will show examples of these two limiting cases.

## **Smectic Membranes in Motion: Surface Diffraction and Dynamic X-ray Scattering**

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We have used smectic membranes as model systems to study low-dimensional ordering and the associated fluctuations. In these systems the divergence of the thermal fluctuations with increasing size of the system prevents the formation of true long-range order (Landau-Peierls instability). Smectic membranes are stacks of liquid layers, which consist of orientationally ordered elongated molecules. They can vary in thickness from two layers (about 5 nm) to thousands of layers (tens of  $\mu\text{m}$ ). In addition they can be made with a high degree of uniformity (mosaicity of a few mdeg), while the lateral dimensions may extend to many cm. I will discuss two examples of studies of smectic membranes with synchrotron radiation.

We have used grazing incidence x-ray diffraction to study the in-plane structure of the top surface layers in smectic membranes of the compound 4O.8. These films show successive layer-by-layer crystallization from smectic-A (Sm-A) to crystalline B (Cr-B) in the exterior layers via an intermediate hexatic smectic-B phase (Sm-B) not observed in the bulk. In a seven-layer membrane we observe at the first step a relatively narrow diffraction peak from the outermost hexatic layers on top of a broad liquid peak from the five liquid interior layers. The Sm-A–Sm-B phase transition in a single top layer turns out to be continuous, while the Sm-B–Cr-B transition is weakly first order.

X-ray photon correlation spectroscopy has been used in a reflectivity geometry to study the dynamics of the layer-displacement fluctuations. Time scales have been pushed down well below microseconds with first measurement points around 10 ns. Fluctuations with long wavelengths reveal an oscillatory damping, while fluctuations with a shorter wavelength show a simple exponential decay. The crossover wave vector  $q_{\perp c}$  scales with the membrane thickness  $L$  as  $q_{\perp c} \sim L^{-1/2}$  making exponential relaxation more pronounced in thick films. Fluctuations of a particular wavelength have been probed by measuring at off-specular scattering positions.

## **In-situ Studies of Biomimetic Thin Films**

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Two important aspects of biogenic mineral nucleation are the kinetics and the organic template, which together can determine the crystal polytype and morphology. Nucleation from a liquid subphase onto a surfactant monolayer is an important model system, since the template charge and lattice spacing may be tuned through control of the surfactant and the surface pressure. Studies up to now have relied upon optical and electron microscopy, and many questions regarding biomineralization are still unresolved, in part because no in-situ probe on atomic length scales has been available.

Using synchrotron x-ray scattering, we have made the first in-situ observations of biomimetic calcium carbonate growth, from its inception as calcium ions collected at a monolayer interface, to a macroscopic amorphous mineral film. With this technique we can quantify the monolayer structure, cation binding, film density and crystallinity as mineralization proceeds, without disturbing the system or interrupting growth. Determination of crystal polytype, orientation, and domain size is also straightforward.

We will describe observations of amorphous mineral precursor films, nucleating at a fatty acid monolayers assembled on a supersaturated calcium bicarbonate subphase containing polyacrylic acid as an inhibitor. At early times, we detect only the monolayer, incorporating a partial layer of bound calcium ions. Subsequently, a film grows at a constant rate to a thickness of about 30 nm, 18 hours later. The film density, which remains constant throughout this growth, is 80% that of calcium carbonate hexahydrate. This suggests that the mineral film has a hydrated, open structure. In-plane diffraction from the monolayer remained unchanged during the 18 hour interval, with no additional peaks appearing at calcite or vaterite Bragg peak positions. However, after 20 hours, intensity from the monolayer Bragg peaks began to shift as crystallization began.

In this system, we find that the polymer concentration and molecular weight drastically affect film growth. However, there is no strong evidence for interactions between the in-plane monolayer structure and that of the mineral film. Hence, this system is probably kinetically, rather than template driven. We hope to clarify the role of the template in future work.



## Changes in Biomolecular Conformation Seen by Small Angle X-ray Scattering

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Small angle X-ray scattering (SAXS) has a long tradition of use for observing the size and shape of biomolecules in solution. In recent years, the availability of synchrotron radiation sources has allowed for qualitative improvements in the ability to measure time resolved changes in conformation, such as protein folding or response to ATP hydrolysis[1]. In addition, improved algorithms have been developed for reconstruction of low resolution 3D density map from 1D SAXS profiles[2]. In this talk, we will review recent work on protein and RNA folding, and on 3D reconstruction applied to studies of TRIC/CCT changes driven by ATP hydrolysis and to Fab complexes with prion oligomers. Also, the possibility of application of SAXS to high throughput structural genomics will be briefly discussed[3].

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## **Ordering at Soft-Hard Interfaces**

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The use of hard substrates to grow ordered soft materials has been extensively studied in recent years, but the use of soft surfaces to nucleate hard materials is much more familiar in nature ('biomineralization'). This talk will describe our X-ray studies of both types of phenomena: ordering in molecular liquids and solutions near silicon substrates, and the growth of inorganic structures under ordered fatty acid (Langmuir) monolayers.

This work was done in collaboration with Jan Kmetko, Chungjong Yu and Guennady Evmenenko.

# **X-ray Measurements of the Orientation Distribution in a Lyotropic Liquid Crystal**

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Analysis of the angular distribution of scattered x-rays from nematic samples has been used to measure the orientation distribution function for the past 25 years. The scattering arises from the interparticle interference and in practice angular scans at constant scattering angle are measured to determine the angular distribution. In principle spatial and orientational correlations of the nematic are coupled in an unknown way, which raises the possibility that the x-ray deduced distribution functions are systematically in error.

We present measurements of the angular distribution from lyotropic nematics composed of the virus fd. This molecule has a crystalline structure so it diffracts to high angle where there are no interparticle correlations. We resolve the question of whether there is a systematic error in the x-ray measurements of the angular distribution function by comparing the angular distribution measured from low-angle interparticle scatter where the spatial and angular order are coupled with the angular distribution measured from intraparticle scattering at high angle.

# **New Insights into the Structure and Property Relationships in Natural and Synthetic Nanocomposites by Synchrotron X-ray Study – from Bone to Fire Resistant Materials**

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At the Advanced Polymers Beamline (X27C) in the National Synchrotron Light Source, one of the research thrust areas is nanocomposites comprising a soft condensed matter matrix and inorganic nanosize fillers. In this talk, some new insights into the structure, property and functionality relationships of two distinctly different classes of nanocomposites: natural bone and synthetic clay-polymer hybrids, will be discussed.

Bone is a composite material where inorganic apatite crystals are embedded in an organic collagen matrix. While the structure of the unmineralized collagen matrix is reasonably well understood, many open questions remain about the mineralized composite, especially about the arrangement of the mineral crystals, their nucleation and growth, and their effects on the collagen matrix. These questions have been addressed by simultaneous synchrotron small- and wide-angle x-ray (SAXS/WAXS) scattering, both from the unmineralized collagen matrix and from the mineralized composite. A consistent model of the mineralization in bone is proposed. The mineral crystals are nucleated with a strong correlation to the collagen matrix. This correlation is not disturbed during crystal growth. The crystals grow larger than the free space provided by the existing holes of the ordered collagen structure, causing perturbations in the collagen structure which are in accordance with the experimental SAXS and WAXS observations.

Nanocomposites consisting of clay additives and synthetic polymers can offer simultaneous improvements of thermal stability, reduced flammability as well as mechanical and barrier properties. However, the improvements primarily depend on the degree of exfoliation and the orientation of the clay, which can be quantitatively characterized by in-situ SAXS and WAXD techniques. We have demonstrated that the degree of clay exfoliation could be controlled by the polarity, ionic interactions, molecular weight of the polymer matrix as well as the processing conditions. In a fully exfoliated poly(vinylacetate)-clay system, a solid-like rheological behavior at high temperatures but a liquid-like rheological behavior at low temperatures were seen. The high temperature composite melt was able to yield and flow, exhibiting the characteristics of a physical crosslinked gel which is responsible for the improved flame retardation properties.

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## **Soft-Matter Physics of Biomembranes: Action of Antimicrobial Peptides**

Huey Huang

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Gene-encoded antimicrobial peptides have no known (protein) receptors. The released peptides spontaneously bind to the lipid matrix of cell membranes and selectively kill microorganisms without harming the host cells. Apparently their mechanisms are based on the physics of peptide-membrane interactions. By oriented circular dichroism, we found that each of a variety of peptides has two binding states to a lipid bilayer, the I state and the S state. In the I state, neutron and X-ray scattering showed transmembrane pores. In a stack of multiple-bilayers, the pores become correlated between bilayers due to the hydration force between bilayers. This provides a means for low-resolution structural analysis by synchrotron radiation. In the S state, the elasticity of lipid bilayer comes into play. The transition between the S and the I state can be explained by a membrane thinning effect. We will also mention a study of dynamics by X-ray inelastic scattering.

## **Soft X-ray Spectromicroscopy for Soft Matter Studies**

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Soft x-ray microscopy can be used to image micrometer-thick organic specimens that can be either dry or hydrated. Fresnel zone plate optics allow this to be done at 30-50 nm spatial resolution. One can also make use of near-edge absorption resonances to do chemical state mapping in complex organic specimens. A frequent mode of analysis involves the collection of a series of images across the carbon edge, giving a three-dimensional data set (X, Y, and energy) from which information can be extracted using multivariate statistical analysis. Use of these methods for the study of some example soft matter systems in geochemistry and biology will be described.

## 2D-3D Transitions in Lung Surfactant

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The primary function of lung surfactant is to form monolayers at the alveolar interface capable of lowering the normal surface tension to near zero. To accomplish this process, the surfactant must maintain a coherent, tightly packed monolayer that avoids collapse during expiration. Previous researchers have shown that the positively charged amino-terminal peptide sequence SP-B<sub>1-25</sub> of lung surfactant-specific protein SP-B increases the collapse pressure of palmitic acid (PA), an important anionic component of lung surfactant, and prevents it from being "squeezed out" from the primarily dipalmitoylphosphatidylcholine (DPPC) lung surfactant monolayer. However, the mechanisms involved in the alteration of the PA isotherms, and the interactions of lung surfactant specific proteins with PA and other surfactant lipids with low collapse pressures are still not well understood.

Utilizing different microscopy techniques, we have observed that SP-B<sub>1-25</sub> in simple phospholipid and model lung surfactant monolayers promote the protrusion of folds into the subphase at low surface tensions. The folds remain attached to the monolayer, appear to be identical in composition to unfolded monolayer, and reversibly reincorporated upon expansion. Without SP-B, an unsaturated lipid-rich phase is irreversibly "squeezed-out" of the monolayer at higher surface tensions. These folded reservoirs reconcile how lung surfactant can achieve both low surface tensions upon compression and rapid respreading upon expansion, and have important implications concerning the design of replacement lung surfactants. The 2D to 3D folding transition has also been observed in simple lipid systems, though never in pure DPPC monolayers. The addition of 'wedge' molecules like palmitic acid or hexadecanol to DPPC monolayers, however, yields folding transitions in these mixed lipid monolayers upon collapse. Using x-ray scattering techniques, we have probed how the presence of 'wedge' molecules affects the packing of the lipids. The alteration of the mechanical properties of the monolayer by the presence of the 'wedge' molecules may be responsible for the ability of the mixed film to collapse via folding. A theoretical model for the folding transition will be discussed. The effects of the incorporation of SP-B<sub>1-25</sub>, the N-terminus peptide of lung surfactant specific protein SP-B, on the structure of palmitic acid monolayers has also been determined via X-ray scattering techniques. Our in-plane results indicate that the peptide fluidizes the host monolayer, but does not affect the packing of the ordered phase. This implies that the peptide resides in a disordered phase, leaving the ordered phase enriched in lipid molecules, which agrees well with our optical microscopy data. Out-of-plane data show that the peptide incorporates itself into the host lipid monolayer at an angle of 36° from the interface, with one end protruding past the headgroup region and into the fluid subphase, and the other end embedded in the tail region of the monolayer.

# **Infrared Microspectroscopy for Probing Biomolecular Dynamics, Structure, and Composition**

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Synchrotron infrared light is an ideal source for infrared micro-spectroscopy due to the combination of its high brightness (i.e. flux density) and broadband nature. Through a 10  $\mu\text{m}$  pinhole, a synchrotron source is 100-1000 times brighter than a conventional globar source and the broadband nature of the source is important for performing spectroscopy.

Unlike many x-ray based spectroscopies that were made possible by the advent of synchrotron radiation, infrared spectroscopy has been used for many years without synchrotron radiation. Although conventional IR microspectroscopy has proven extremely valuable for resolving the chemical components in biomaterials, the long wavelengths of infrared light limit the spatial resolution that can be achieved. Existing instruments using a conventional IR source encounter a signal-to-noise (S/N) limitation when apertures confine the IR to an area of 20-30  $\mu\text{m}$  in diameter. The high brightness of the synchrotron source allows smaller regions to be probed with acceptable S/N. Indeed, aperture settings smaller than the wavelength of light can be used; though in this case, diffraction controls the available spatial resolution. Thus for a typical biological specimen, the diffraction-limited spatial resolution for primary lipid (C-H stretch), protein (amide I), and nucleic acid (P-O stretch) absorption features is approximately 3, 6, and 12  $\mu\text{m}$ , respectively. This improvement in spatial resolution achieved by using a synchrotron IR source has only been realized recently, and applications to the dynamics, structure, and composition of biomaterials are still in their infancy.

In this talk, applications of synchrotron infrared micro-spectroscopy to a number of biomaterials will be presented. Topics may include (1) the protein and mineral content in bone during osteoporosis and osteoarthritis, (2) misfolded protein structure in Alzheimer's disease and scrapie, (3) enzyme structure and biopolymerization, (4) chemical changes in single cells during various stages of apoptosis, and (5) the sub-millisecond folding of proteins such as cytochrome c.

This work was performed at Beamlines U10B and U2B at the National Synchrotron Light Source, Brookhaven National Laboratory. Scientific personnel on these projects include: Cathy Carlson (*Univ. of Minnesota*); David Hamerman, Mark Chance, Pam Bromberg, and Raymond Huang (*Albert Einstein College of Medicine*); David Burr (*Indiana Univ.*); Judit Miklossy (*Temple University*) and Lazslo Forro (*Swiss Federal Institute of Technology*); Janina Kneipp and Dieter Naumann (*Robert Koch Institute*); Ying Mei and Richard Gross (*Polytechnic Univ.*); Paul Dumas, Nadege Jamin, and Jean-Luc Teillaud (*LURE and Institute Curie- Paris*). More information on the infrared programs at the NSLS can be found at <http://infrared.nsls.bnl.gov>.

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## **SAXS Applied to Biomolecules in Solution**

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Small angle X-ray scattering in solution, using the intense beam of synchrotron radiation, is in principle a method of choice to monitor the shape, conformational changes and association processes of biomacromolecules, as well as their long-range interactions. In particular, the structural response to any physico-chemical modification of the environment can be easily followed. The principles have become a reality in the last decade mainly thanks to significant improvements in data analysis. A number of software packages are now available to manipulate three-dimensional structural data under the constraint of solution scattering curves. More recently, the development of instruments using the highly collimated and intense beam of undulators at third generation synchrotron facilities has brought along the kinetic dimension in SAXS experiments.

These advances will be presented and illustrated through different biological systems. The main characteristics of the SAXS beam-line pre-project at the future French synchrotron facility SOLEIL will also be shortly introduced.

This work was prepared in collaboration with Patrice Vachette.

## Thin Liquids, X-ray Scattering Studies

Peter S. Pershan

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X-ray reflectivity has proven to be one of the best-suited methods for experimental determination of the atomic level surface structure of surfaces coated by thin films. It has had wide success in the study of thin films on solids. A number of similar experiments of thin films on liquid surface have also been carried out; however, these encounter the problem of separating thermally induced roughness from the intrinsic structure. We will review some of the principle ideas behind this problem and present new theoretical results that should simplify the solution. The results will be illustrated with new data for thin wetting films of perfluoromethylcycloheane (PFMH)  $C_6F_{11}CF_3$  that coat the surface molten alkane ( $CH_3(CH_2)_{18}CH_3$ , C20).

## **Novel Liquid-Crystal Orders in Unusual Contexts**

L. Radzihovsky

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Liquid-crystal phases, understood as states of matter intermediate in their properties between fully disordered isotropic liquids and fully ordered crystals are ubiquitous in nature. I will discuss a variety of systems that exhibit liquid-crystal order in somewhat unusual contexts. These will include novel phases of smectic liquid crystals confined inside low-density aerogels, melted two-dimensional colloidal crystals in the presence of a periodic potential, polymerization dynamics in a smectically-ordered host, and nonequilibrium smectic order in randomly pinned, driven systems. Common themes of the importance of thermal fluctuations and external perturbations, as well as utility of x-ray scattering to study the resulting states will be emphasized.

## **Patterning Polymers From the Nano to the Micro**

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Electric fields have been shown to be a very effective means of obtaining alignment in thin polymer films containing domains that are anisotropic in shape. The essential difference between bulk and thin film behavior is the energy required to overcome differences in the interfacial energies of the components. Results indicate that alignment in block copolymers and layered polymers occurs by an amplification of density fluctuations at the interface between these nanoscopic domains. A pressure, developed at the interface, essentially pulls the domains into alignment. This phenomenon is shown to map from the nanoscopic level with copolymers to the microscopic level with polymer bilayers where microscopic domain alignment is found. X-ray and neutron scattering and reflectivity have prove to be indispensable tools for the characterization of the alignment mechanism and ordering. These aligned morphologies provide a unique means of producing arrays of nanoscopic structures that are finding use in a wide range of applications ranging from biological to magnetic storage devices.

# DNA-Lipid Complexes and Interactions with Cells: Supramolecular Assembly and Gene Delivery

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There is now a surge of activity in developing nonviral cationic-based gene delivery systems for therapeutic applications [1], in part, because of their nonimmunogenicity and ease of production, but also because the single largest advantage of nonviral over viral methods for gene delivery is the potential of transferring extremely large pieces of DNA into cells. This was demonstrated when partial fractions of order 1 Mega base pairs of human artificial chromosome was recently transferred into cells using cationic lipids (CLs) as a carrier although extremely inefficiently [2]. We will describe recent work on the self-assembled structures of CL-DNA complexes by the quantitative techniques of synchrotron x-ray diffraction. Distinct structures have been discovered including, a multilamellar structure with alternating lipid bilayer and DNA monolayers [3], an inverted hexagonal structure with DNA coated by cationic lipid monolayers and arranged on a two-dimensional lattice [4], and lamellar phases containing polypeptides and cytoskeletal filamentous actin [5]. We describe recent confocal optical imaging and cell transfection results which are beginning to unravel the relationship between these distinct nanostructured supramolecular assemblies, including key physical and chemical parameters of the lipid-carriers, and how effectively DNA gets transported into cells [6]. Supported by NIH and NSF.

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## Molecular Ordering at Liquid-Liquid Interfaces

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X-ray reflectivity, x-ray diffuse scattering, and interfacial tension measurements are used to probe molecular ordering at the water-alkane interface under a variety of conditions.

(1) Reflectivity studies of the neat interface for alkane carbon numbers varying from 6 to 22 reveal a significant difference between the measured interfacial width and values predicted from capillary wave theory or from computer simulations. The variation of interfacial width with carbon number can be described by combining the capillary-wave prediction with a contribution from intrinsic structure. This intrinsic structure is determined by the gyration radius for the shorter alkanes and by the bulk correlation length for the longer alkanes.

(2) Soluble monolayers of hydrogenated and fluorinated n-alcohols are adsorbed at the water-hexane interface. X-ray reflectivity is used to determine the molecular ordering in the solid monolayer phase that occurs at low temperatures. The hydrogenated monolayers have a distinctive type of disorder at low temperatures. The monolayer thickness is the same as a molecular length, indicating that the molecules are oriented nearly perpendicular to the interface and are nearly all-trans. Penetration of hydration water molecules into the region of the head group must be accompanied by head group disorder along the interfacial normal. The region of the tail group next to the head group is nearly close-packed while the region adjacent to the hexane is more disordered. This disorder is in contrast to the very ordered, close-packed monolayers formed by fluorinated alcohol surfactants at the water-hexane interface.

(3) The alcohol monolayers undergo a solid to gas phase transition as a function of temperature. The monolayers form domains near the transition temperature. X-ray off-specular diffuse scattering is used to directly probe the statistical distribution of domains in monolayers of the fluorinated alcohol. The domains have a nearly constant radius of 1.5 micrometers and a nearest neighbor separation that varies from 2 to 8 micrometers with temperature. Evidence that the domains are of equilibrium size suggests that the domain line tension can be determined from these measurements.

## **Synchrotron X-ray Studies of the Statics and Dynamics of Fluctuations at the Surfaces of Polymer Films**

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Studies of surface (capillary wave) fluctuations on polymer films using the techniques of x-ray scattering have yielded a number of intriguing results over the last few years and indicate that, unlike the surfaces of simple liquids, the dynamics of polymer surfaces and interfaces need to be understood in greater detail. In particular, issues such as interaction with the substrate, chain entanglement effects, and the approach to the glass transition all affect the dynamics at the surface. We shall review some recent results on polystyrene films of different thicknesses and at different temperatures obtained from studies of off-specular diffuse (static) scattering (which measures the static height-height correlation function) and also using the relatively new technique of X-Ray Photon Correlation Spectroscopy (XPCS) which is the analogue of Dynamical Light Scattering and which measures the dynamical height-height correlation function. Both these techniques extend the lateral length-scales, which can be probed to at least an order of magnitude smaller than those observable with light scattering.

We shall discuss to what extent these results can be described by the ordinary capillary wave theory for liquid surfaces.

This work was done in collaboration with H.J. Kim (U.California San Diego), A. Ruhm (MPF, Stuttgart), L.B. Lurio (Northern Illinois University), S.G.J. Mochrie (Yale University), J.K. Basu (U.Illinois Urbana-Champaign), J. Lal (Argonne National Laboratory)

## **Anomalous X-ray Scattering Study on Counterion Condensed DNA**

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Because they are negatively charged, DNA molecules repel each other in monovalent salt solutions. However, in multivalent salt solutions [e.g. Mn, Co], DNA molecules attract one another and subsequently condense into liquid crystalline arrays. Nature makes use of these attractive interactions when packing DNA into confined spaces, such as those found in sperm heads and virus capsids. Established models of the electrostatic double layer, such as the Poisson-Boltzmann mean-field approach, fail to predict attraction between identically charged surfaces. Current speculation attributes such attraction to density fluctuations of the ionic atmospheres surrounding the DNA molecules, which give rise to a van der Waals-type interaction, or to the attraction brought about by a Wigner crystal-like arrangement of counterions on adjacent DNA strands. Therefore, an understanding of the spatial arrangement of counterions around DNA molecules in multivalent salt solutions is necessary to evaluate such hypotheses.

We used anomalous x-ray scattering at the Advanced Photon Source (Argonne National Labs, IL), to measure of the counterion distribution around DNA molecules. These distributions should change as environmental conditions are changed from condensing to non-condensing, according to the aforementioned hypotheses.



## **Anomalous Diffraction at the $M_V$ Edges of Uranium and Bismuth and at the K Edges of Chlorine, Sulphur and Phosphorus**

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X-ray diffraction at wavelengths beyond 2 Å is very much triggered by the use of anomalous dispersion in many kinds of structural studies. Two features of anomalous dispersion are of interest:

1. the increase of the imaginary part of the resonant atomic scattering amplitude with the square of the wavelength (method of single wavelength anomalous diffraction, SAD).
2. the presence of strong anomalous dispersion of some M-edges of heavy elements, like U and Th, and of the anomalous dispersion near K-edges of light elements like Cl, S, and P which are newcomers in multi-wavelength anomalous dispersion techniques (MAD, DAFS).

While (1) allows a bargaining between strength of the anomalous signal and the influence of absorption and the reduction resolution one is ready to accept, there is no similar choice with MAD techniques using the absorption edges mentioned in (2). The properties of soft X-rays have to be considered carefully. Two aspects are most relevant:

- the decrease of the penetration depth of soft X-rays with the inverse of the cube of the wavelength in soft condensed matter.
- the limit of structural resolution, which is given by half the wavelength if one allows for scattering angles up to back reflection.

With an eventually increased sensitivity to radiation damage of the sample the simultaneous measurement of the diffraction pattern over a large solid angle is mandatory, e.g., in macromolecular crystallography using wavelengths close to the K edge of sulphur (5.02 Å) or that of phosphorus (5.76 Å).

As air is a severe absorber of soft X-rays, two ways of instrumentation for soft X-ray diffraction are being used: one of them relies on an almost completely evacuated beamline from the source to the detector. The other option is a helium atmosphere between the beam exit and the detector. An evacuated beamline has been used at HASYLAB till end of 1996. There were no windows between the housing of the diffractometer ( $p < 0.01$  mb) and the high vacuum section of the beamline. The sample was kept in a small plastic dome of 3 mm diameter filled with nitrogen cooled by a Peltier cascade down to -110°C. Four multi-wire proportional counters filled with Ar/CO<sub>2</sub> with a sensitive area of 300 mm x 300 mm covering scattering angles between -60° to 120° were supplied by the EMBL Outstation Grenoble. This beam line produced excellent diffraction patterns from various protein and ribosome crystals at wavelengths near the K-edge of sulphur and phosphorus [1]. Extensive studies of small-angle scattering from solutions (e.g. SAXS from P in ribosomes) and on purple membrane preceded the single crystal work. The HASYLAB set up would have needed considerable further development for routine operation.

Since 1998 test experiments of soft X-ray diffraction were started at the beamline ID1 of ESRF. After the elimination of air gaps and absorbers the fine structure of anomalous diffraction (DAFS) of a chlorobismuthate single crystal was measured at 30 wavelengths near the K edge of chlorine and the  $M_V$  edge of bismuth [2]. The sample crystal and the diffractometer were in *vacuo* (no windows upstream). Much of the diffracted intensity was absorbed by the 250 µm beryllium window of the CCD camera. Temperature control of the sample is less readily established.

## Spatially Resolved Reflectivity Measurements of Wetting Dynamics

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We have probed the dynamics of precursing thin film growth and the structure of the region between a bulk meniscus and the thin film. The structures are formed on vertical, silicon dioxide on silicon substrates by dipping an edge of the substrate into a viscous polymeric liquid, 1000 cSt polydimethylsiloxane. We perform spatially resolved x-ray reflectivity using a small spot scanned across the surface. We characterize the shape of the bulk liquid/vapor interface by straightforward beam blocking measurements. In the transition and thin films regions we use careful fitting of specular reflection curves to obtain the thickness, density, and roughness of the fluid layer. In the transition region, where the liquid is  $\sim 10^2$  Å thick, we find anomalously large roughness that is a significant fraction of the film thickness. The thickness of the fluid layer decreases rapidly with height and about 0.5 mm above the contact line the layer transforms to a monolayer that exhibits the density of the bulk fluid.

# **Self-Assembly Phases Of Biopolymers, Membranes, Proteins & Counterions**

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We describe the structure and interactions of a novel class of biomolecular self-assemblies, where new condensed phases of various biopolymers are formed through their interactions with oppositely charged ions of varying complexity, from point-like multivalent ions to charged amphiphilic molecules. Intuitively, two like-charged macromolecules in aqueous solution are expected to repel one another, which is essentially the prediction of prevailing mean-field theories. In the presence of oppositely charged multivalent ions, however, many biopolymers actually attract one another and condense into compact, ordered states. We have examined the global phase behavior of a large range of charged biopolymers: DNA, cytoskeletal F-actin, Fd and M13 viruses. For example, we unambiguously demonstrate the existence of two distinct condensed phases in F-actin. At low multivalent ionic strengths, a homogeneous liquid of uncondensed filaments is observed. At high multivalent concentrations, the filaments condense into uniaxial bundles, in the form of close-packed parallel arrays of individual filaments. At intermediate multivalent concentrations, however, we find a new phase of liquid crystalline matter, in the form of a multi-axial network. In contrast, cationic amphiphilic molecules can condense F-actin into hierarchically organized tubules with no direct analog in simple membrane systems. Using high resolution synchrotron small angle x-ray scattering, confocal microscopy and electron microscopies, we will present a systematic structural investigation of these condensed biopolymer phases, and the resultant implications for our understanding of polyelectrolytes, as well as a range of outstanding biomedical problems.